Virology 418 (2011) 21–26

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Lysine acetylation sites in bovine foamy virus transactivator BTas are important for its DNA binding activity

Rui Chang [1], Juan Tan [1], Fengwen Xu, Hongqi Han, Yunqi Geng, Yue Li, Wentao Qiao *

Key Laboratory of Molecular Microbiology and Biotechnology (Ministry of Education), College of Life Sciences, Nankai University, Tianjin 300071, China
Key Laboratory of Microbial Functional Genomics (Tianjin), College of Life Sciences, Nankai University, Tianjin 300071, China

ARTICLE INFO

Article history:
Received 1 December 2010
Returned to author for revision 3 June 2011
Accepted 7 July 2011
Available online 3 August 2011

Keywords:
p300
Bovine foamy virus
BTas
Acetylation

ABSTRACT

Cellular acetylation signaling is important for viral gene regulation, particularly during the transactivation of retroviruses. The regulatory protein of bovine foamy virus (BFV), BTas, is a transactivator that augments viral gene transcription from both the long terminal repeat (LTR) promoter and the internal promoter (IP). In this study, we report that the histone acetyltransferase (HAT), p300, specifically acetylates BTas both *in vivo* and *in vitro*. Further studies demonstrated that BTas acetylation markedly enhances its transactivation activity. Mutagenesis analysis identified three lysines at positions 66, 109 and 110 in BTas that are acetylated by p300. The K110R mutant lost its binding to BFV promoter as well as its ability to activate BFV promoter. The acetylation of K66 and K109 may contribute to increased BTas binding ability. These results suggest that the p300-acetylated lysines of BTas are important for transactivation of BFV promoters and therefore have an important role in BFV replication.

Introduction

Acetylation of core histone and non-histone proteins is a key process in the regulation of diverse intracellular pathways, including chromatin remodeling and modulation of protein functions (Chen et al., 2001; Glozak et al., 2005; Sterner and Berger, 2000). Acetylation of the protein substrates is catalyzed by histone acetyltransferases (HATs), a large family of structurally and functionally related proteins, including p300, CBP, and PCAF. Recent studies have shown that cellular acetylation is one of the primary targets of viral proteins during their life cycle, which affects gene expression of both virus and the host (Caron et al., 2003; Col et al., 2002; Hottiger and Nabel, 2000). The viral proteins of human immunodeficiency virus type 1 (HIV-1) (Cereseto et al., 2005; Deng et al., 2000; Kiernan et al., 1999; Ott et al., 1999), Human T-lymphotropic virus type 1 (HTLV-1) (Lodewick et al., 2009), and the prototype foamy virus (PFV) (Bannert et al., 2004; Bodem et al., 2007) are substrates of HATs, and acetylation leads to a functional advantage for these viral proteins. In addition to retroviruses, viruses of other families (Gwack et al., 2001; Wang et al., 2000; Zhang et al., 2006) are known to interact with different HATs. These observations suggest that acetylation of the viral proteins is a ubiquitous strategy for the regulation of virus replication.

Foamy viruses (FVs), also known as *spumavirus*, are complex retroviruses in the *Spumaretrovirinae* subfamily of *Retroviridae* (Delelis et al., 2004; Linial, 1999). Unlike conventional retroviruses, FVs bear a second promoter between *Tas* and the 3′-end of *env*, termed the internal promoter (IP), in addition to the long terminal repeat (LTR) (Linial, 1999). The FV Tas protein is the central regulator in viral gene expression and the key switch between latent and lytic infection (Meiering and Linial, 2002). The HATs p300 and PCAF specifically interact with PFV Tas protein *in vivo*, resulting in enhancement of Tas-dependent transcriptional activation (Bannert et al., 2004). Subsequently, the Tas of both PFV and feline foamy virus (FFV) is proposed to be acetylated by PCAF, leading to increased promoter-binding ability (Bodem et al., 2007).

Bovine foamy virus (BFV) is a non-primate member of *Spumaretrovirinae* that was isolated from cattle in 1983 (Johnson et al., 1983). Genomic analyses revealed similar sequence properties between BFV and PFV. The BTas protein is the transactivator that enhances viral transcription through binding to viral promoters, and its response elements have been mapped (Tan et al., 2010). BTas is a 249-amino acid regulator that contains one N-terminal DNA-binding domain and one C-terminal activation domain, and the dimerization is required for its transactivational activity (Tan et al., 2008, 2010).

Whether BTas associates with HATs has not been thoroughly investigated. In this paper, our data suggested that p300 specifically acetylates BTas both *in vivo* and *in vitro*. p300 and BTas synergistically activated the BFV LTR and IP promoters. Three lysines at positions 66, 109, and 110 in the binding domain of BTas are identified as sites for p300-dependent acetylation. These lysines are essential to transactivation and binding ability of BTas. Moreover, acetylation increases the affinity of wild-type BTas to its responsive DNA elements. In

* Corresponding author at: College of Life Sciences, Nankai University, 94 Weijin Rd., Tianjin 300071, China. Fax: +86 22 23501783.
E-mail address: wentaoqiao@nankai.edu.cn (W. Qiao).
[1] These authors contributed equally to the work.

0042-6822/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2011.07.003

conclusion, we report the involvement of HATs and the significance of acetylated lysines in BTas-dependent transactivation of BFV.

Results

p300 acetylates BTas and regulates BTas-dependent transactivation

To investigate whether acetylation is relevant for BTas transactivation, we used a histone deacetylase inhibitor, trichostatin A (TSA) to inhibit histone deacetylase, and found that BFV promoters were stimulated by treating BTas transfected-cells with TSA. Moreover, BFV replicated more efficiently in TSA-treated cells, suggesting that BTas may interact with HATs or undergo acetylation itself (see Supplementary 1 for details). To demonstrate whether HATs acetylate BTas and which histone acetyltransferases are functional, 293T cells were transfected with plasmids expressing BTas and HAT candidates (p300, CBP, and GCN5). BTas was immunoprecipitated from the cell lysates, separated by SDS-PAGE, and blotted with anti-acetyl lysine antibody to detect acetylation modification. The results suggest that p300, but not CBP or GCN5, specifically acetylates BTas protein *in vivo* (Fig. 1A). To confirm that BTas is a substrate for p300, we performed *in vitro* acetylation experiment using recombinant HAT domain (1077–1718 aa) of p300. As a positive control, histone H3 was strongly acetylated by the p300 HAT domain (Fig. 1B). Using this *in vitro* assay system, the recombinant His-tag BTas protein was incubated with p300 HAT and Ac-CoA, and then analyzed for acetylation. The results indicated that BTas is a substrate for the HAT activity of p300 and can be acetylated by p300 *in vitro* (Fig. 1C).

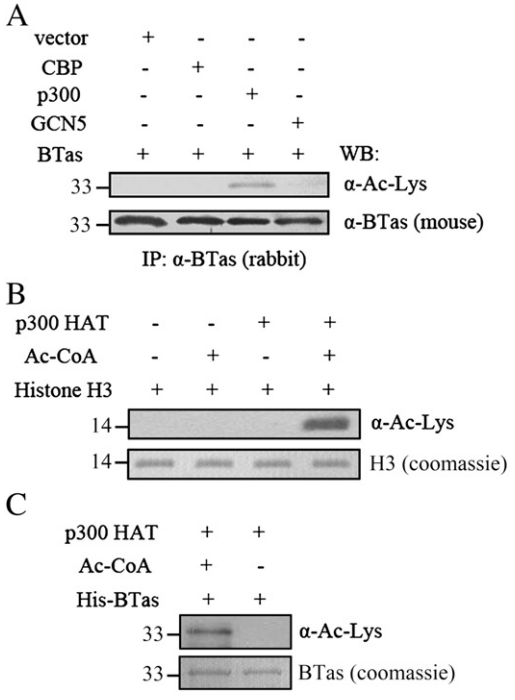

Fig. 1. BFV BTas is acetylated by histone acetyltransferase p300 *in vivo* and *in vitro*. (A) p300 acetylates BTas *in vivo*. pCMV-BTas construct were transiently transfected into 293T cells with CBP, p300, GCN5, or an empty vector. BTas protein was immunoprecipitated with rabbit anti-BTas antibody and immunoblotted with anti-acetylated lysine antibody (upper panel). The same membrane was then striped and re-probed with mouse anti-BTas antibody (lower panel). (B) *In vitro* acetylation of histone H3. Histone H3 was incubated with p300 HAT domain or Ac-CoA, or both, at 37 °C for 60 min as indicated. Samples were then subjected to Western analysis using anti-acetylated lysine antibody (upper panel). The histone H3 protein used in the assay was detected by Coomassie blue staining (lower panel). (C) p300 acetylates BTas *in vitro*. Purified His-tagged BTas was incubated with p300 HAT domain and Ac-CoA at 37 °C for 60 min. Samples were then subjected to Western blotting using anti-acetylated lysine antibody (upper panel). The His-BTas protein used in the assay was detected by Coomassie blue staining (lower panel).

To examine the involvement of p300 in BTas-mediated transactivation, 293T cells were co-transfected with pCMV-BTas, luciferase expression plasmid driven by BFV LTR or IP, and increasing amounts of p300. Luciferase activity was measured 48 h later. As shown in Fig. 2, BTas-mediated activation of LTR and IP promoters were both enhanced by p300 in a dose-dependent manner. Based on these findings, we conclude that p300 is the functional acetyltransferase for BTas acetylation, and that p300 and BTas can synergistically transactivate BFV promoters.

Identification of the lysine residues in BTas that are acetylated by p300

Next, we determined the specific sites of BTas that were acetylated by p300. A series of BTas N-terminal deletions were expressed as GST-BTas fusion proteins, and analyzed using *in vitro* HAT assay for their acetylation (Fig. 3A). The results demonstrated that p300 efficiently acetylates the D1 deletion mutant, whereas the D2 deletion mutant was acetylated to a much lesser extent (the acetylation level was approximately 25% of that observed with D1). The acetylation of D3 was, however, barely detectable (approximately 8% acetylation compared to D1) (Fig. 3B, left panel, lane 4). Equivalent amounts of substrate proteins were loaded in each lane as shown by Coomassie blue staining (Fig. 3B, right panel). Taken together, the acetylation targets of BTas recognized by p300 may locate in cluster 2 (62–89 aa) and cluster 3 (90–113 aa). The lysine residues of these regions were individually replaced by arginine and their potential to activate BFV promoter were measured. The K66R, K74R, K109R and K110R mutations led to impaired activation (see Supplementary 2 for details). Lysines 109 and 110 were agreed with the "G/S K motif"

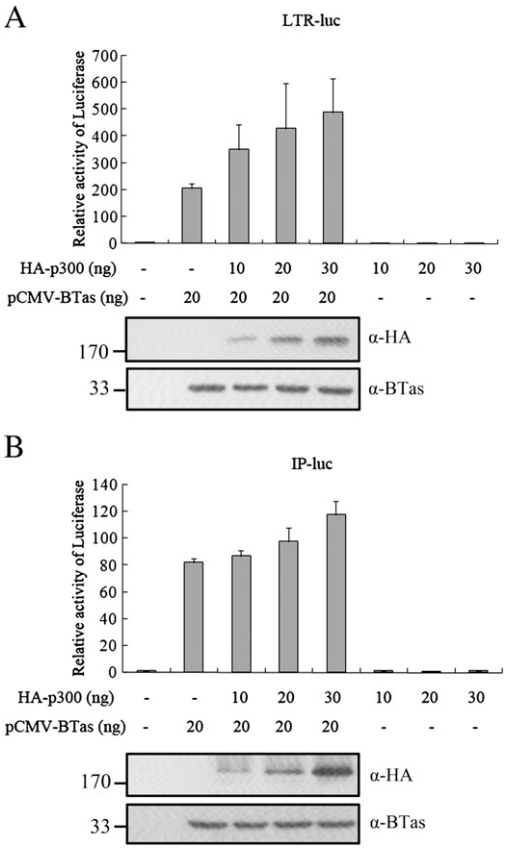

Fig. 2. p300 synergizes with BTas to transactivate the BFV LTR and IP promoters. (A) 293T cells were transfected with pCMV-BTas and pBFV LTR-Luc in the absence or presence of increasing amounts of HA-p300. Cellular extracts were assayed for luciferase activity at 48 h post-transfection. (B) The same experiment as in (A) was carried out except for transfection with pBFV IP-Luc instead of pBFV LTR-Luc. The results are representative of three independent transfections.

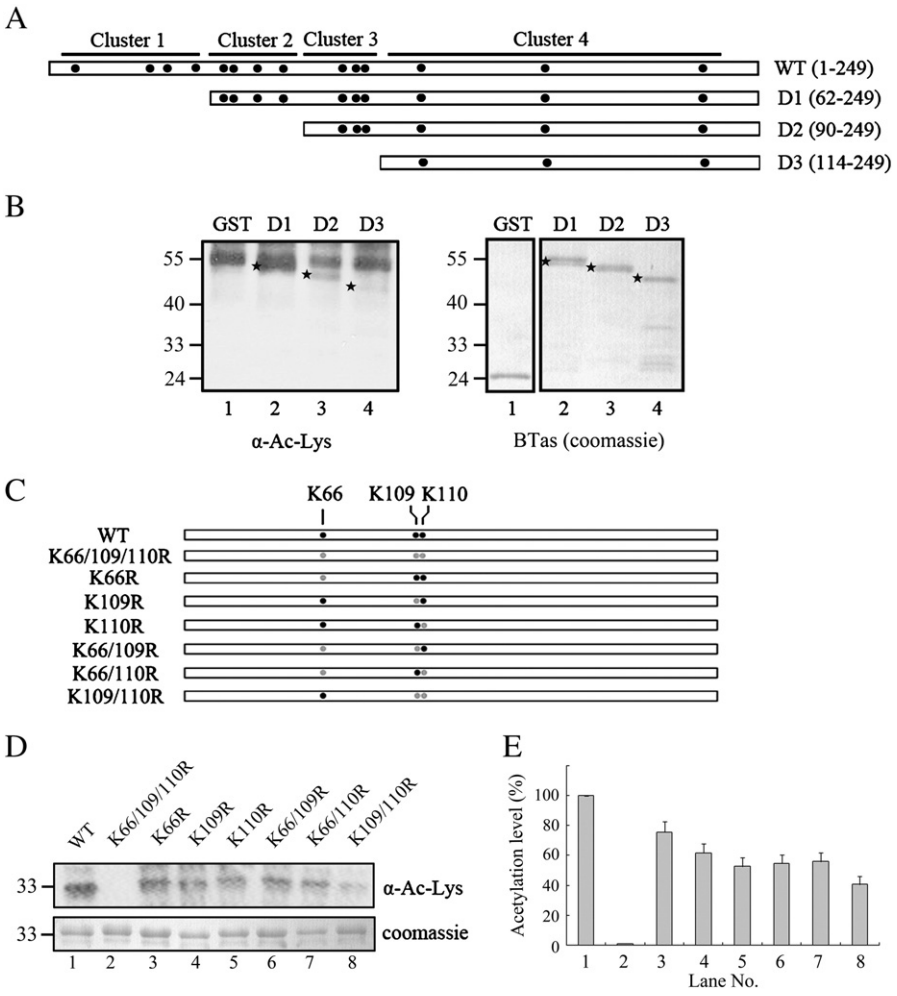

Fig. 3. Identification of acetylated lysines in BTas. (A) Schematic representation of BTas N-terminal truncations. Lysines are indicated by black dots. (B) *In vitro* acetylation of BTas N-terminal truncations by p300. Recombinant GST-tagged BTas N-terminal truncations, or GST were incubated with p300 HAT and Ac-CoA. The reactions were subjected to Western analysis using anti-acetylated lysine antibody (left-hand) or Coomassie blue staining (right-hand). (C) Schematic representation of BTas point mutants at amino acid positions 66, 109, and 110. Lysines and arginines are indicated by black and gray dots, respectively. (D) *In vitro* acetylation of BTas lysine point mutants by p300 HAT. Recombinant wild-type His-BTas and lysine point mutants were incubated with p300 HAT domain and Ac-CoA as described above. The reactions were subjected to Western analysis using anti-acetylated lysine antibody (upper panel) or Coomassie blue staining (lower panel). (E) The acetylation levels of His-BTas and lysine point mutants were quantified by ImageJ software and shown as columns.

(see Discussion for details). Besides, K66 was identified as target for p300-dependent acetylation by mass spectrometry (data not shown). Taken together, these results indicated that K66, K109 and K110 maybe potential sites for p300-mediated acetylation.

To examine this hypothesis, lysines at amino acid positions 66, 109, and 110 were changed into arginines (Fig. 3C). These mutants were incubated with p300 HAT and *in vitro* acetylation assays were performed. The data revealed that p300 acetylates BTas when single or double lysines were replaced by arginines (40% to 75% that of wild-type) (Figs. 3D and E, lanes 3–8). The triple mutation (K66/109/110R), however, completely abrogated p300-dependent acetylation (Figs. 3D and E, lanes 2). This result confirms that K66, K109, and K110 of BTas are acetylated by p300.

Acetylation of BTas increases its DNA-binding ability

Since acetylation is known to regulate the protein function, we investigated whether acetylation of the three lysines is essential for BTas nuclear translocation. We found that wild-type BTas and the triple mutant both localized to the nucleus, suggesting that the three lysines were not necessary for the nuclear import of BTas (see Supplementary 3 for details).

Further, since the acetylation of PFV and FFV Tas protein increase their promoter-binding ability, we determined whether acetylation was important for the DNA binding affinity of BTas. To address this issue, electrophoretic mobility shift assays (EMSA) were carried out. As shown in Fig. 4A (left panel), increasing amounts of purified His-tagged BTas were incubated with p300 HAT, and *in vitro* HAT assays were performed. The acetylated and mock-acetylated BTas were then incubated with a probe corresponding to the BFV LTR.TRE. The DNA–protein complexes were analyzed by EMSA. As expected, a shifted BTas-DNA complex was clearly detected at higher protein concentrations (Fig. 4A, left panel, lane 4), whereas acetylated BTas bind to the LTR at the modest protein concentration (Fig. 4A, left panel, lane 6). The binding affinity of acetylated BTas was elevated compared to their counterparts in the unmodified BTas group (Fig. 4B and compare lanes 5–7 with lanes 2–4 in Fig. 4A, left panel). The GST protein was unable to bind BFV LTR.TRE (Fig. 4A, right panel, lane 1), and the binding was abolished when excess unlabeled LTR.TRE was added (Fig. 4A, right panel, lane 3), demonstrating the specific interaction between BTas and LTR. These results suggested that the DNA-binding property of BTas was increased by p300-mediated acetylation. To further address the requirement of BTas acetylation for DNA binding, wild-type His-BTas or triple mutant were *in vitro* acetylated and tested for their DNA-binding affinity by EMSA. Acetylation enhanced BTas affinity to

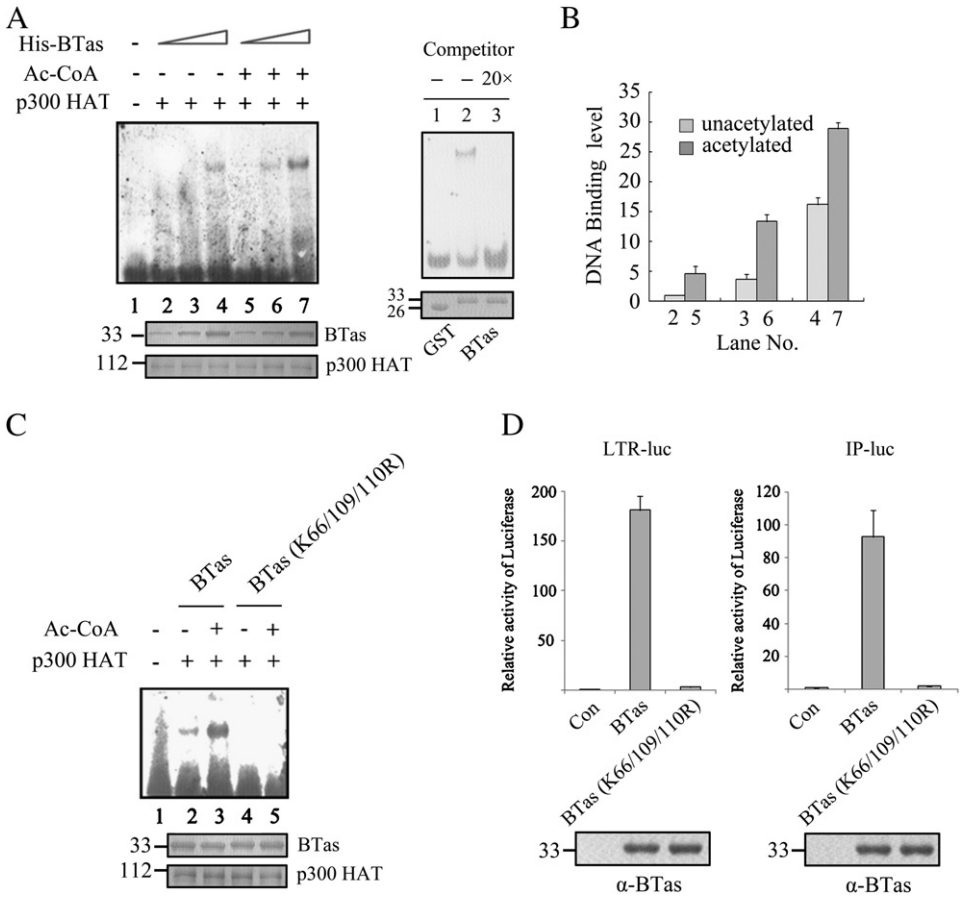

Fig.4. Acetylation of BTas promotes its DNA-binding ability. (A) Acetylation of wild-type BTas enhances DNA-binding affinity. Increasing amounts of recombinant His-BTas (200 ng, 500 ng and 1 μg) were incubated with p300 HAT domain in the presence or absence of Ac-CoA and *in vitro* acetylation was carried out as described above. The acetylated and mock-acetylated BTas were then incubated with digitonin-labeled BFV LTR.TRE and subjected to EMSA. The BTas and p300 HAT domain used in the assay were detected by Coomassie blue staining and silver staining, respectively (lower panel). In the competition assay (right panel), GST was used as binding protein (lane 1), and His-BTas was incubated with BFV LTR in the absence (lane 2) or presence (lane 3) of 20-fold excess of unlabeled BFV LTR. The GST and BTas protein used in the assay were detected by Coomassie blue staining (lower panel). (B) The binding levels of BTas corresponding to each lane were quantified by ImageJ software, and indicated as columns. (C) Wild-type and triple lysine-substituted BTas were *in vitro* acetylated or mock acetylated, then incubated with the BFV LTR.TRE probe and subjected to EMSA. The BTas and p300 HAT domain used in the assay were detected by Coomassie blue staining and silver staining, respectively (lower panel). (D) BTas-dependent transactivation requires acetylated lysines. 293T cells were transfected with wild-type BTas or the triple lysine mutant, in the presence of pBFV LTR-Luc or pBFV IP-Luc. Cellular extracts were examined for luciferase activity at 48 h post-transfection. The results are representative of three independent transfections.

the DNA targets by about four fold (Fig. 4C, lane 3). However, binding of the BTas triple mutant to the DNA targets was completely abolished (Fig. 4C, lane 4). Moreover, we found that wild-type BTas efficiently transactivated LTR and IP promoters, while the triple mutation at K66, K109, and K110 led to impaired transactivation (Fig. 4D). These data illustrated that these lysines are necessary for BTas-mediated transcription. Besides, acetylation of one or more of these residues may be relevant to the enhancement of BTas DNA binding affinity.

Acetylated lysines of BTas are important for DNA binding ability of BTas

We next investigated whether K66, K109 and K110 play a key role in the binding of BTas to DNA. To this end, in addition to the triple-lysine mutant as described above, the three lysines were changed to arginine either individually or in combinations of two. DNA binding ability of these mutants was measured by EMSA. The results showed that mutation of K110, either individually or in combination of K66 or K109, abrogated BTas/DNA interaction (Fig. 5A). We then performed *in vitro* p300-mediated acetylation experiment to determine whether acetylation of K66 and K109 contributes to BTas/DNA binding. The results showed that acetylation of wild-type BTas increased its affinity for DNA (Fig. 5B, lane 3). Although the K66/109R mutant exhibited a DNA binding ability similar to that of wild-type BTas (Fig. 5B, lane 4), acetylation reaction did not increase the DNA affinity of this mutant (Fig. 5B, lane 5). Together, our data demonstrate the key role of K110 in BTas/DNA interaction. Moreover, the enhancing effect of BTas binding maybe partly results from the acetylation of K66/K109.

Discussion

The transactivator BTas is the central regulatory protein in BFV replication, however, little is known about the mechanisms underlying its regulation. In this study, we have used plasmid cotransfection assay, *in vitro* acetylation assay and EMSA to investigate the role of acetylation in BTas transactivation. Our data showed that p300 cooperates with BTas to enhance the transactivation activity of the BFV LTR and IP promoters. The acetylation of BTas can increase its DNA binding affinity, and the K66, K109, and K110 are critical residues for the DNA binding ability of BTas. Thus, we hypothesized that acetylation of BTas protein by p300 and the acetylated lysines of BTas are important in the regulation of BTas-mediated transcription and virus–host interaction.

Recent investigations indicate that a number of viral proteins are substrates for cellular histone acetyltransferase. For instance, the HIV-1 transactivator Tat was acetylated by p300 at lysine 50, which facilitates separation of the Tat-TAR complex and enhances transcription of the integrated provirus (Kiernan et al., 1999). The acetylation of HIV integrase by p300 increased its DNA-binding ability and integration activity (Cereseto et al., 2005). In HTLV-1, the crosstalk

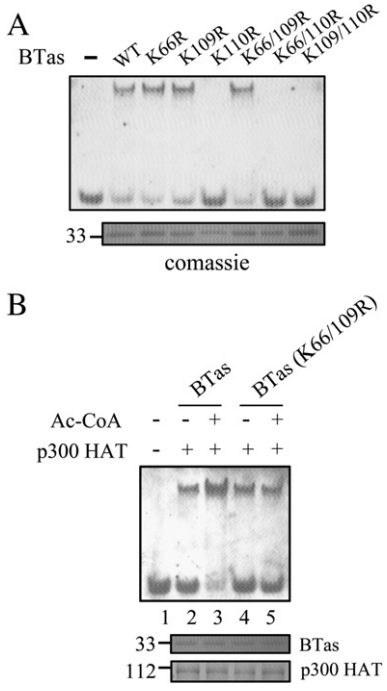

Fig.5. Acetylated lysines are important for DNA-binding ability of BTas. (A) Wild-type and mono- or double-lysine-substituted BTas were incubated with BFV LTR.TRE and subjected to EMSA. The proteins used in the assay were detected by Coomassie blue staining (lower panel). (B) Wild-type BTas and K66/109R mutant were *in vitro* acetylated or mock acetylated, then incubated with the BFV LTR.TRE probe and subjected to EMSA. The BTas and p300 HAT domain used in the assay were detected by Coomassie blue staining and silver staining, respectively (lower panel).

between Tax and p300 has a profound effect on activation of the NF-κB signaling pathway (Lodewick et al., 2009). The Tas protein of PFV functionally interacts with p300 and PCAF, and can be acetylated by PCAF (Bannert et al., 2004; Bodem et al., 2007). Thus, our findings are similar to the results obtained with these retroviruses. By contrast, two members of the HAT family, GCN5 and CBP, do not acetylate BTas. Therefore, it is possible that p300 is the most common acetyltransferase used by retroviruses.

To date, there have been no reports regarding the acetylated sites in FV Tas; thus, we set out to search for acetylated lysines in BTas by p300-mediated acetylation. The three acetylated residues, K66, K109, and K110, all locate in the binding domain (1–133 aa) of BTas, which is consistent with the finding that p300 acetylates lysines in the DNA/RNA-binding domain of HIV-1 integrase and Tat (Cereseto et al., 2005; Deng et al., 2000; Kiernan et al., 1999; Ott et al., 1999). Regulation by acetylating lysines in the binding domain is a phenomenon observed in many cellular regulators (Martinez-Balbas et al., 2000; Masumi et al., 1999; Thevenet et al., 2004). Of note, if a lysine is preceded directly by a glycine, a serine or another lysine, these two residues constitute the "G/SK motif". The latter lysine, in some cases, served as potential site for p300-dependent acetylation (Bannister et al., 2000; Wolf et al., 2002). Not all of the acetylated lysines exhibit the G/SK sequence. However, the K109 and K110 of BTas are indeed found in such a motif.

Acetylation affects a variety of protein functions, including DNA-binding capability, transactivation ability, protein stability, subcellular localization, and protein–protein interaction (Chen et al., 2001; Glozak et al., 2005). Our current study revealed a synergistic activation of BFV promoters by p300 and BTas. This result is consistent with the observation that p300 promotes Tas and Tat-mediated transcription in PFV and HIV-1 (Bannert et al., 2004; Ott et al., 1999). Previous studies demonstrated that acetylation of FFV Tas increases its DNA binding, while Tat was dissociated from TAR by K50-acetylation (Bodem et al., 2007; Kiernan et al., 1999). In this paper, we showed that acetylation promotes BTas DNA-binding ability, which was similar to FFV Tas. We further found that the triple lysine mutant was unable to bind viral promoter. We could not exclude the possibility that the substitution of three lysines to arginines alters other functions, such as binding property or conformation, of wild-type BTas. Thus, the mono- and double-lysine mutants were tested in EMSA. The data demonstrated that DNA binding was abrogating once K110 was mutated. It is probably that the dissociation of the triple mutant with DNA was due to the absence of K110. Further results confirmed that the DNA binding ability of K66/109R mutation was not significantly enhanced by acetylation. Based on these observations, we assume that the increased DNA binding affinity of BTas maybe partly dependent on the acetylation of K66 and K109. In contrast, it is likely that the lysine at position 110 is associated with the DNA binding property of BTas. Since the acetylation of different lysines in Tat regulates different functions (Col et al., 2001; Kiernan et al., 1999), the mechanism of BTas acetylation at these lysines needs further in-depth investigation.

In the present study, we demonstrated that the acetylation of BTas by p300 facilitates its interaction with the BFV promoters, thus recruiting transcriptional partners to activate viral transcription. This finding suggests that acetylation is a ubiquitous mechanism adopted by foamy viruses to regulate gene expression. The current study shed light on virus–host interactions and the mechanisms of BFV replication through deciphering the relationship between BTas and p300, as well as their interactions with the basal transcription machinery.

Materials and methods

Cell culture, virus, transfection and reporter gene assay

293T cells were maintained in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum, 50 U/ml penicillin, and 50 μg/ml streptomycin sulfate. BFV indicator cell line (BFVL) was established by transfection of BHK21 cells with luciferase expression gene that was driven by the BFV LTR promoter. G418 was added to select resistant colonies. The virus titer of BFV was measured by BFV LTR directing luciferase expression by coculture cells infected with BFVL. All cells were cultured in 10% CO_2 atmosphere at 37 °C. Bovine foamy virus 3026 was isolated from peripheral lymphoid of a BIV seropositive cattle by our lab (Liu et al., 1997).

Transfection was performed by using polyethyleneimine (Sigma) (Durocher et al., 2002) and corresponding plasmids. Transfection of pCMV β-gal was carried out for normalization of transfection efficiency. Cells were lysed and luciferase activity was measured 48 h post-transfection according to the manufacturer's protocol (Promega). All the transfection experiments were carried out at least three times.

Plasmids and recombinant proteins

pCMV-BTas, pBFV LTR-Luc and pBFV IP-Luc have been described previously (Liu et al., 1999; Zhang et al., 2000). The histone acetyltransferase expression plasmid HA-p300, CBP and GCN5 were obtained from Upstate. The sequence coding for the C-termini of BTas (amino acids 62 to 249, 90 to 249 and 114 to 249) were generated by PCR using pCMV-BTas as template. The products were cloned into pGEX-6p-1(Amersham Biosciences) vector to construct GST-tagged BTas truncations. The plasmid for expression of His-BTas was prepared by subcloning of BTas cDNA from pCMV-BTas into pET32a vector (Novagen). The lysine mutants were generated by PCR-based site directed mutagenesis (QuickChange Kit, Stratagene) using His-BTas as template. The p300 HAT domain expression plasmid was constructed by inserting a PCR-amplified fragment encoding amino acids 1077–1718 of p300 cDNA into pGEX-6P-1 vector. All the new constructs were verified by DNA sequencing.

Expression constructs for GST-p300 HAT, GST-tagged BTas truncations, His-tagged BTas and lysine mutants were transformed into *E. coli*

strain BL21 (DE3). Purification were carried out by glutathione-agarose affinity chromatography (Amersham Biosciences) or Ni-nitrilotriacetic acid-agarose chromatography (Novagen) according to the manufacturer's protocols. Protein concentrations were determined by Bradford method.

Antibodies and immunoprecipitation assay

Anti-acetylated-lysine mAb were purchased from Cell Signaling Technology. The polyclonal antibody against BTas was prepared by immunization of rabbit or mouse according to a standard procedure. Immunoprecipitations were carried out using lysate prepared from 293T cells that were transfected with pCMV-BTas and different HATs expression plasmids. Briefly, 48 h after transfection, cells were harvested and washed with ice cold PBS, lysed in 1 ml of lysis buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 1% NP-40, and 1 mM phenylmethylsulfonyl fluoride). After sonication, the cell debris was removed by centrifugation at 10,000 *g* for 15 min at 4 °C. The supernatant was incubated with rabbit anti-BTas antibody at 4 °C for 2 h. The immune precipitates were retrieved with 25 μl of Protein A-Sepharose (50% slurry, Sigma-Aldrich) for another 2 h at 4 °C. Then the immune complexes were washed six times with lysis buffer. The immunocomplexes were boiled in 2× Laemmli sample buffer and fractionated with 12% SDS-polyacrylamide gel, transferred to Immobilon-P Transfer Membrane (Millipore), and blotted with appropriate antibodies. Analysis of chemiluminescent signals was performed with Western Blotting Luminol Reagent (Santa Cruz Biotechnology). The intensity of bands was analyzed using ImageJ software and corrected by subtracting the background noise.

In vitro acetylation assay

In vitro acetylation assays were performed using 1 μg of substrate protein, 500 ng of bacteria purified p300 HAT domain, and 10 μM of Ac-CoA (sigma) in HAT buffer (50 mM Tris–HCl, pH 7.5, 10% glycerol, 0.1 mM EDTA, 50 mM KCl, 10 mM sodium butyrate, 1 mM dithiothreitol and 1 mM phenylmethylsulfonyl fluoride). The proteins of 30 μl final volume were incubated at 37 °C for 60 min. Samples were then denatured in 2× Laemmli sample buffer and resolved by 12% SDS-PAGE. The gels were stained with Coomassie brilliant blue or subjected to Western blotting using anti-acetylated-lysine antibody.

Electrophoretic mobility shift assay

EMSA was carried out using DIG Gel Shift Kit (Roche). Briefly, oligonucleotide corresponding to BFV LTR.TRE −186 to −165 (5′-ATAGCTATTTTAGTAAGTTAGC-3′) was synthesized and annealed with the complementary strand. The purified native and acetylated BTas were incubated with DIG-labeled probe in binding buffer for 20 min at room temperature, the DNA–protein complexes were separated on 6% non-denaturing polyacrylamide gel and subjected to visualization by anti-digoxigenin-AP antibody according to the protocol.

Acknowledgments

This work was supported by grants from the Chinese Ministry of Health (2008ZX 10001-002), the 973 program (2010CB534907), the National Natural Science Foundation of China (30900068 and 81071343), and the 111Project (B08011). We gratefully thank Professor Chen Liang (Micgill University, Canada) for the writing assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.virol.2011.07.003.

References

Bannert, H., Muranyi, W., Ogryzko, V.V., Nakatani, Y., Flugel, R.M., 2004. Coactivators p300 and PCAF physically and functionally interact with the foamy viral transactivator. BMC Mol. Biol. 5, 16.
Bannister, A.J., Miska, E.A., Gorlich, D., Kouzarides, T., 2000. Acetylation of importin-alpha nuclear import factors by CBP/p300. Curr. Biol. 10 (8), 467–470.
Bodem, J., Krausslich, H.G., Rethwilm, A., 2007. Acetylation of the foamy virus transactivator Tas by PCAF augments promoter-binding affinity and virus transcription. J. Gen. Virol. 88 (Pt 1), 259–263.
Caron, C., Col, E., Khochbin, S., 2003. The viral control of cellular acetylation signaling. Bioessays 25 (1), 58–65.
Cereseto, A., Manganaro, L., Gutierrez, M.I., Terreni, M., Fittipaldi, A., Lusic, M., Marcello, A., Giacca, M., 2005. Acetylation of HIV-1 integrase by p300 regulates viral integration. EMBO J. 24 (17), 3070–3081.
Chen, H., Tini, M., Evans, R.M., 2001. HATs on and beyond chromatin. Curr. Opin. Cell Biol. 13 (2), 218–224.
Col, E., Caron, C., Seigneurin-Berny, D., Gracia, J., Favier, A., Khochbin, S., 2001. The histone acetyltransferase, hGCN5, interacts with and acetylates the HIV transactivator, Tat. J. Biol. Chem. 276 (30), 28179–28184.
Col, E., Gilquin, B., Caron, C., Khochbin, S., 2002. Tat-controlled protein acetylation. J. Biol. Chem. 277 (40), 37955–37960.
Delelis, O., Lehmann-Che, J., Saib, A., 2004. Foamy viruses—a world apart. Curr. Opin. Microbiol. 7 (4), 400–406.
Deng, L., de la Fuente, C., Fu, P., Wang, L., Donnelly, R., Wade, J.D., Lambert, P., Li, H., Lee, C.G., Kashanchi, F., 2000. Acetylation of HIV-1 Tat by CBP/P300 increases transcription of integrated HIV-1 genome and enhances binding to core histones. Virology 277 (2), 278–295.
Durocher, Y., Perret, S., Kamen, A., 2002. High-level and high-throughput recombinant protein production by transient transfection of suspension-growing human 293-EBNA1 cells. Nucleic Acids Res. 30 (2), E9.
Glozak, M.A., Sengupta, N., Zhang, X., Seto, E., 2005. Acetylation and deacetylation of non-histone proteins. Gene 363, 15–23.
Gwack, Y., Byun, H., Hwang, S., Lim, C., Choe, J., 2001. CREB-binding protein and histone deacetylase regulate the transcriptional activity of Kaposi's sarcoma-associated herpesvirus open reading frame 50. J. Virol. 75 (4), 1909–1917.
Hottiger, M.O., Nabel, G.J., 2000. Viral replication and the coactivators p300 and CBP. Trends Microbiol. 8 (12), 560–565.
Johnson, R.H., Oginnusi, A.A., Ladds, P.W., 1983. Isolations and serology of bovine spumavirus. Aust. Vet. J. 60 (5), 147.
Kiernan, R.E., Vanhulle, C., Schiltz, L., Adam, E., Xiao, H., Maudoux, F., Calomme, C., Burny, A., Nakatani, Y., Jeang, K.T., Benkirane, M., Van Lint, C., 1999. HIV-1 tat transcriptional activity is regulated by acetylation. EMBO J. 18 (21), 6106–6118.
Linial, M.L., 1999. Foamy viruses are unconventional retroviruses. J. Virol. 73 (3), 1747–1755.
Liu, S., Chen, H., Chen, J., Liang, D., Chen, Q., Geng, Y., Wood, C., 1997. Isolation and identification of a bovine spuma virus isolate 3026. Chin. J. Virol. 13 (2), 140–145.
Liu, J., Liu, S., Chen, Q., Geng, Y., 1999. Borf-1 protein identified as a transcriptional transactivator of bovine foamy virus. Chin. Sci. Bull. 44, 1017–1021.
Lodewick, J., Lamsoul, I., Polania, A., Lebrun, S., Burny, A., Ratner, L., Bex, F., 2009. Acetylation of the human T-cell leukemia virus type 1 Tax oncoprotein by p300 promotes activation of the NF-kappaB pathway. Virology 386 (1), 68–78.
Martinez-Balbas, M.A., Bauer, U.M., Nielsen, S.J., Brehm, A., Kouzarides, T., 2000. Regulation of E2F1 activity by acetylation. EMBO J. 19 (4), 662–671.
Masumi, A., Wang, I.M., Lefebvre, B., Yang, X.J., Nakatani, Y., Ozato, K., 1999. The histone acetylase PCAF is a phorbol-ester-inducible coactivator of the IRF family that confers enhanced interferon responsiveness. Mol. Cell. Biol. 19 (3), 1810–1820.
Meiering, C.D., Linial, M.L., 2002. Reactivation of a complex retrovirus is controlled by a molecular switch and is inhibited by a viral protein. Proc. Natl. Acad. Sci. U.S.A. 99 (23), 15130–15135.
Ott, M., Schnolzer, M., Garnica, J., Fischle, W., Emiliani, S., Rackwitz, H.R., Verdin, E., 1999. Acetylation of the HIV-1 Tat protein by p300 is important for its transcriptional activity. Curr. Biol. 9 (24), 1489–1492.
Sterner, D.E., Berger, S.L., 2000. Acetylation of histones and transcription-related factors. Microbiol. Mol. Biol. Rev. 64 (2), 435–459.
Tan, J., Qiao, W., Xu, F., Han, H., Chen, Q., Geng, Y., 2008. Dimerization of BTas is required for the transactivational activity of bovine foamy virus. Virology 376 (1), 236–241.
Tan, J., Hao, P., Jia, R., Yang, W., Liu, R., Wang, J., Xi, Z., Geng, Y., Qiao, W., 2010. Identification and functional characterization of BTas transactivator as a DNA-binding protein. Virology 405 (2), 408–413.
Thevenet, L., Mejean, C., Moniot, B., Bonneaud, N., Galeotti, N., Aldrian-Herrada, G., Poulat, F., Berta, P., Benkirane, M., Boizet-Bonhoure, B., 2004. Regulation of human SRY subcellular distribution by its acetylation/deacetylation. EMBO J. 23 (16), 3336–3345.
Wang, L., Grossman, S.R., Kieff, E., 2000. Epstein–Barr virus nuclear protein 2 interacts with p300, CBP, and PCAF histone acetyltransferases in activation of the LMP1 promoter. Proc. Natl. Acad. Sci. U.S.A. 97 (1), 430–435.
Wolf, D., Rodova, M., Miska, E.A., Calvet, J.P., Kouzarides, T., 2002. Acetylation of beta-catenin by CREB-binding protein (CBP). J. Biol. Chem. 277 (28), 25562–25567.
Zhang, L., Qiao, W., Liu, S., Wang, J., Chen, Q., Geng, Y., 2000. Regulatory elements that mediate transactivation by Tas in the internal promoter of bovine foamy virus. Chin. J. Virol. 16, 232–236.
Zhang, Y., Jiang, Y., Geiser, V., Zhou, J., Jones, C., 2006. Bovine herpesvirus 1 immediate-early protein (bICP0) interacts with the histone acetyltransferase p300, which stimulates productive infection and gC promoter activity. J. Gen. Virol. 87 (Pt 7), 1843–1851.